# *SALMONELLA* PREVALENCE IN PIGS REARED ON FARMS WITH AND WITHOUT ANTIMICROBIALS

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**Abstract** A convenience sample of farms using antimicrobials (antimicrobial-using, AMU) post-weaning for therapy and/or growth promotion (n=21) were contrasted to a convenience sample of farms not using antimicrobials post-weaning (antimicrobial-free, AMF, n=21) distributed across three US geographic areas. Fecal samples were screened for *Salmonella enterica* prior to slaughter and carcasses were tested after slaughter. Fecal samples were collected from 30 pigs per farm. At slaughter, 10 carcasses were swabbed at each of three locations on the processing chain. *Salmonella spp*. were isolated using conventional methods. The proportion of *Salmonella* positive fecal samples among AMF farms was higher (27.3%, CI 12.8-41.8%) when compared with AMU farms (12.3%, CI 5.0-19.5%). No differences were found among carcass swabs or antibiograms. It is concluded that AMF production may not enhance *Salmonella* food safety in the pork chain.



**Introduction** Antimicrobials have been commonly used in commercial swine production. In the US, 88.5% of producers reported using antimicrobials in the feed at least once in the six months prior to the survey (USDA, 2002). Although commonly used, the effects of antimicrobials on the shedding of *Salmonella enterica* have not been quantified in commercial US settings. Logically, the application of antimicrobials could reduce the prevalence or duration of *Salmonella* shedding (Ebner, *et al.*, 2000, Evangelisti, *et al.*, 1975). Conversely, shedding of antimicrobial resistant *Salmonella* strains could be enhanced when antimicrobials are provided *Salmonella*-positive pigs.

As part of a multi-state study to determine the prevalence, antimicrobial resistance and genotypic diversity of three major foodborne pathogens in swine, *Salmonella, Campylobacter* and *Yersinia,* the goal of this report was to compare *Salmonella spp.* in market swine reared either on farms using or avoiding antimicrobials in growing pigs. The larger study includes 60 farms sampled in three distinct geographic areas in the US in North Carolina, Ohio/Michigan and the portions of the upper Midwest (South Dakota, Iowa, Minnesota and Wisconsin).

**Methods** A total of 42 farms were selected, distributed over three geographic locations in the U.S: North Carolina, Ohio-Michigan, and South Dakota, Iowa, Minnesota and Wisconsin. Antimicrobials were provided in the feed on 21 farms for at least part of the post-weaning period, and 21 avoided antimicrobials post-weaning. In the latter group, pigs requiring antimicrobial therapy were treated individually and were then removed from the population at the time of treatment and not re-introduced to the population.

Fecal samples (10 g) were collected from each of 30 pigs on each farm within 72 hours of slaughter. In the case of small farms marketing less than 30 animals on a single day, all pigs destined for slaughter were sampled. At slaughter, sterile 8.9 x 12.7 cm cellulose swabs were used to sample skin surfaces. These were 3x larger area than the official USDA carcass swabs to allow the swabs to be divided after collection and distributed to the three participating laboratories. Shortly before evisceration 10 carcasses were swabbed (swab A) and the same 10 carcasses were sampled post-evisceration (swab B). After chilling, one swab was collected from each side of 10 carcasses on either side (i.e., left and right sides) of the carcass. These swabs were taken from carcasses not sampled previously to avoid sampling from the same surface. The first of these post-chilling samples, swab C, was collected in the same manner as swabs A and B. Swab D was collected from the opposite side using the published USDA: FSIS methods (Federal Register, 1996), except that both the swab and template were the larger size described above. A 300 $cm^2$ area was sampled in each of three locations, on the ham, lateral to the ventral midline, and on the neck using a sanitized template. In cases where the jowl area had less than 300 $cm^2$ available for sampling the collector approximated 300 $cm^2$ in an irregular pattern. In total, 40 swabs were taken for each farm.

Fecal samples were enriched in tetrathionate broth (37°C, 48 hours); 100 µl was transferred to R-10 after 48 h. After incubation of 18-24 hours, samples were streaked for isolation on XLT-4

agar. Suspect colonies were streaked for isolation on BG agar. Confirmation of *Salmonella spp.* identity was confirmed by agglutination via multivalent *Salmonella* antisera (Polyvalent O groups A through G *Salmonella* Somatic Agglutinating Serum, Rabbit, Remel, Lenexa, KS, USA). Carcass swabs were processed as described by the FSIS methods for carcasses, (Federal Register, 1996) except that XLT4 agar was substituted for DMLIA agar.

Antibiograms were determined for isolates from fecal samples using the Sensititre system (TREK Diagnostic Systems, Inc., Cleveland, Ohio, USA) with a customized plate containing 17 antimicrobials. Minimum inhibitory concentrations (MIC) cut-points are described the NCCLS (National Committee for Clinical Laboratory Standards, 1999).

The association between antimicrobial use and detection of *Salmonella* was tested in logistic regression models adjusted for expected herd-level clustering. The associations between antimicrobial use and antimicrobial resistance were evaluated using PROC MIXED for MIC and PROC GLIMMIX for the proportion of isolates resistant to antimicrobials. Prevalence estimates were calculated using PROC SURVEFREQ (SAS Institute, Cary, NC, USA), with CI adjusted for the expected cluster effect of herd of origin. Prevalence and 95% CI intervals were calculated by sample, and for samples with significant differences, by herd type.



**Results** Pigs from AMF farms had increased odds of *Salmonella* detection in farm collected fecal samples compared with AMU farms, (OR 2.4, CI 1.5-3.7, p<0.01). No differences were found for carcass swabs. The proportion of *Salmonella*-positive fecal samples among AMF farms was 27.3% (CI 12.8-41.8%) and 12.3% (CI 5.0-19.5%) for AMU farms. For carcass swabs, the proportion of *Salmonella*-positive samples was as follows across both farm types: A, 9.2% (CI 3.1-15.3%), B, 9.9% (CI 5.5-14.3%), C, 3.9% (CI 0-8.0%), D, 5.0% (CI 1.7-8.3%). No differences in carcass swab positive status were detected between the farm categories.

Antimicrobial resistance did not significantly differ between isolates from the two farm types for any antimicrobial tested. The proportion of resistance detected was as follows, in declining order: Tetracycline, 65.1% (CI 38.3-91.9%), streptomycin, 23.5% (CI 6.5-40.5%), amoxicillin/clavulanic acid 22.8% (CI 5.6-40.1%), ampicillin 22.1% (CI5.4-38.9%), chloramphenicol 16.8% (CI 0.0-34.0%), sulfafizoxazole 4.0% (CI 0.0-9.0%), cefoxitin 4.0% (CI 0.0-11.5%) trimethoprim/sulphamethoxazole 1.3% (CI 0.0-3.1%) and kanamycin 1.0% (CI 0.0-3.5%). No resistant isolates were detected for amikacin, ceftriaxone, ciprofloxacin, gentamicin, nalidixic acid or ceftiofur.

**Discussion** The major findings of this study are that, relative to conventional farms using antimicrobials after weaning, pigs from AMF farms 1) had a 2.4 fold increased odds of *Salmonella* in live, slaughter-ready pigs, 2) had no difference in *Salmonella* on carcasses, and 3) had no differences in antimicrobial resistance in *Salmonella* detected in feces. Taken together, these findings suggest that AMF production may not provide improved food safety regarding either the potential for exposure to *Salmonella enterica* in the pork chain, or with regard to the potential for exposure to antimicrobial resistant strains of *Salmonella*. Consequently, post production techniques to avoid public health risks in all phases of the pork chain need to be considered equally for AMF and AMU production systems.

To our knowledge, this is the first report of *Salmonella* prevalence in commercial US AMF farms. The increased risk for *Salmonella* detection on AMF farms leaves open the possibility that antimicrobials may suppress *Salmonella* shedding on AMU farms. It must be noted, however, that the AMU and AMF farms were a convenience sample of farms, and consequently the findings here do not necessarily reflect that of all farms. However, these farms were selected without knowledge of prior *Salmonella* status, and without reference to other characteristics other than the convenience of sampling and the use of antimicrobials. It was clear from observations of the farms that many characteristics varied between the production types, so it is likely that the differences in *Salmonella* shedding may in part be attributable to factors other than the simply the use/non-use of antimicrobials. These findings appear to justify further work to more fully understand the relationship between antimicrobial use and *Salmonella* shedding.

The lack of differences in antibiograms may suggest that, once selected in larger pig populations, antimicrobial resistance is persistent. Alternately, pigs on ABF farms may have continued exposure to non-pig sources of antimicrobial resistant *Salmonella*, including feed, wildlife and/or feral animals and other sources. Without strong selective pressures to favor antimicrobial sensitive strains, it is possible that antimicrobial resistant strains can persist for extended periods, as

has been demonstrated in a study of an AMF farm that avoided antimicrobials for 28 years (Reeves, *et al.*, 2002).

**Conclusions** Relative to conventional antimicrobial using farms, antimicrobial free farms produced pigs that had

- 2.4 fold higher risk for *Salmonella* shedding in fecal samples collected 24-48 hours prior to slaughter
- No differences in *Salmonella* isolation rates on carcass swabs collected at slaughter
- No differences in antimicrobial resistance among 17 antimicrobials tested

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